

Claims:

1. A plasmid vector for targeted transformation of filamentous fungi comprising
 - a) an origin of replication for a host organism not originating from the filamentous fungi to be transformed;
 - b) a selection marker for a host organism not originating from the filamentous fungi;
 - c) a promotor facilitating recombinant expression in fungi that is functionally linked to the coding region of the hygromycin resistance gene which is functionally linked to a terminator which facilitates transcription termination in filamentous fungi;wherein the overall size of the elements a) , b) and c) does not exceed 4500 bp;
and
 - d) a nucleic acid sequence which is homologous to nucleic acid sequences of the filamentous fungi to be transformed and makes homologous recombination in the filamentous fungi to be transformed possible.
2. A plasmid vector as claimed in claim 1, wherein the origin of replication a) originates from bacteria.
3. A plasmid vector as claimed in claims 1 to 2, wherein the selection marker b) imparts a resistance to antibiotics.
4. A plasmid vector according to claims 1 to 3, wherein the promotor of element c) is selected from the group consisting of the GPD-1-, PX6-, TEF-, CUP1-, PGK-, GAP1-, TPI, PH05-, AOX1, GAL10/CYC-1, CYC1, OliC-, ADH-, TDH-, Kex2-, MFa- and the NMT-promotor.

5. A plasmid vector according to claims 1 to 4, wherein the terminator of element c) is selected from the group consisting of the AOX1-, nos-, PGK-, TrpC- and the CYC1-terminator.
6. A plasmid vector according to claims 1 to 5, wherein the promotor of element c) is the GPD-1-promotor and the terminator of element c) is the nos-terminator.
7. A plasmid vector according to claims 1 to 6, wherein the nucleic acid sequence d) is functionally linked to a promoter facilitating recombinant expression in filamentous fungi.
8. A plasmid vector according to claims 1 to 7, wherein the nucleic acid sequence d) is functionally linked to a transcription terminator facilitating recombinant expression in filamentous fungi.
9. A selection marker for the targeted transformation of filamentous fungi according to any of claims 1 to 8 comprising a nucleic acid sequence encoding a polyketide synthetase fragment, wherein said nucleic acid sequence comprises
 - i. a nucleic acid sequence shown in SEQ ID NO:1 or SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:5; or
 - ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or
 - iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 47% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10; or

- iv. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp; or
- v. parts of the nucleic acid sequence as defined in i., ii. or iii. consisting of at least 300bp comprising

a) a nucleic acid sequence shown in SEQ ID NO:7 ; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:8 by back translation; or

c) a functional equivalent of a nucleic acid sequence set forth in a) , which is encoded by amino acid sequence that has at least an identity of 89% with the SEQ ID NO:8.

10. Use of a nucleic acid sequence comprising

a) a nucleic acid sequence encoding a polyketide synthetase; or

b) parts of the nucleic acid sequence as defined in i. consisting of at least 300bp

as marker for targeted transformation in filamentous fungi.

11. Use of a nucleic acid sequence according to claim 10 said nucleic acid sequence comprising

i. a nucleic acid sequence according to claim 9; or

ii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11; or

iii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

- iv. a functional equivalent of the nucleic acid sequence set forth in i) , which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 38% with the SEQ ID NO:6 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:8 that has at least an identity of 39% with the SEQ ID NO:8 or from a functional equivalent of an amino acid sequence shown in SEQ ID NO:10 that has at least an identity of; or
 - v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp;or
 - vi. parts of the nucleic acid sequence as defined in ii., iii or iv. consisting of at least 300bp comprising a nucleic acid sequence, which is encoded by an amino acid sequence that has at least an identity of 68% with the SEQ ID NO:8.
12. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising a selection marker comprising a nucleic acid sequence encoding a polyketide synthetase fragment, said nucleic acid sequence comprising
- i. a nucleic acid sequence according to claim 9; or
 - ii. a functional equivalent of the nucleic acid sequence set forth in i) , which is encoded by an amino acid sequence that has at least an identity of 40% with the SEQ ID NO:6; or
 - iii. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;
 - iv. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation; or

v. parts of the nucleic acid sequence as defined in ii., iii. or iv. consisting of at least 300bp; or

vi. parts of the nucleic acid sequence as defined in i., ii. or iii. or iv. consisting of at least 300bp comprising a nucleic acid sequence, which is encoded by a functional equivalent of an amino acid sequence that has at least an identity of 68% with the SEQ ID NO:8.

13. An expression cassette comprising

a) a promotor sequence in functional linkage with a nucleic acid sequence according to claim 9 in antisense orientation; and optionally

b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).

14. A plasmid vector for targeted transformation of filamentous fungi as claimed in claims 1 to 8, additionally comprising an expression cassette according to claim

13.

15. An expression cassette comprising

a) a promoter sequence in functional linkage with a nucleic acid sequence comprising

i. a nucleic acid sequence shown in SEQ ID NO: 3, 4 or 5; or

ii. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:6 by back translation; or

iii. a functional equivalent of the nucleic acid sequence set forth in i) which is encoded by an amino acid sequence shown in SEQ ID NO:6 that has at least an identity of 40% with the SEQ ID NO:6; or

iv. a nucleic acid sequence shown in SEQ ID NO:9 or SEQ ID NO: 11;

v. a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:13 by back translation;

and optionally

b) further genetic control sequences functionally linked to a nucleic acid sequence according to a).

16. A method for transforming filamentous fungi, comprising the following steps

a) transferring a plasmid vector according to claim 12 or 14 into a filamentous fungi;

b) selecting successfully transformed filamentous fungi by the absence of color.

17. A method for transformation of filamentous fungi, comprising the following steps

a) providing a filamentous fungi, in which the polyketide synthetase gene is modified in such away that the polyketide synthetase cannot be functionally expressed;

b) transforming the filamentous fungi of step a) with an expression cassette according to claim 15 or a vector comprising the aforementioned expression cassette;

c) selecting successfully transformed filamentous fungi by the presence of color.

18. A method as claimed in claims 16 or 17, wherein the plasmid vector comprises at least an additional selection marker.
19. A method as claimed in any of claims 16 to 18 wherein the selection is confirmed by PCR.
20. A method as claimed in any of claims 16 to 19, wherein the filamentous fungi are successfully transformed and identified in a high-throughput screening.